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16. ABSTRACT

Trimethyltin has been reported to produce morphological alterations in the brain which are primarily restricted to the limbic system. A variety of physiological measures of limbic system integrity are discussed in terms of their ability to detect TMT-induced dysfunction. In addition, several measures of sensory system dysfunction are discussed. It is concluded that limbic system dysfunction induced by this compound is detected more efficiently by intrahippocampal evoked potentials than by more gross measures of dysfunction. It is also concluded that relying upon preliminary descriptions of pathological alterations to direct physiological studies may provide an incomplete description of neurotoxicity.

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Physiological Methods for Assessment of Trimethyltin Exposure

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DYER, R. S. *Physiological methods for assessment of Trimethyltin exposure.* NEUROBEHAV TOXICOL TERATOL 4(6) 659–664, 1982.—Trimethyltin has been reported to produce morphological alterations in the brain which are primarily restricted to the limbic system. A variety of physiological measures of limbic system integrity are discussed in terms of their ability to detect TMT-induced dysfunction. In addition, several measures of sensory system dysfunction are discussed. It is concluded that limbic system dysfunction induced by this compound is detected more efficiently by intrahippocampal evoked potentials than by more gross measures of dysfunction. It is also concluded that relying upon preliminary descriptions of pathological alterations to direct physiological studies may provide an incomplete description of neurotoxicity.

TMT Trimethyltin Organotin Evoked potentials Hippocampus Neurotoxicity Kindling Afterdischarge Visual evoked potentials Somatosensory potentials

NEUROPHYSIOLOGICAL methods are most often used to evaluate mechanisms of brain function. In the context of toxicology, neurophysiological methods may be chosen which are useful for assessing dysfunction of a particular system, or for assessing the functional mechanism of dysfunction. For example, the visual evoked potential recorded from the cortex may reveal dysfunction of the visual system, but will not accurately characterize the nature of that dysfunction. Intracellular and extracellular recordings from visual system neurons can reveal the contributions of membrane properties and synaptic inputs to the general dysfunction revealed by the gross recordings. The present paper focuses upon gross measures of brain function for detection of neurotoxicity to different functional systems (in this case, sensory and limbic). As a vehicle for discussion, the methods are considered in terms of their ability to detect dysfunction induced by trimethyltin (TMT).

TMT has recently become a compound of interest to neurotoxicologists (e.g. [3, 4, 5]). Two reports have described the consequences of human exposure to TMT. In the first [14] two chemists were exposed to a mixture of dimethyl and trimethyltin chloride. The major symptoms described were mental confusion, seizures, and various psychic disturbances (e.g., memory defects, loss of vigilance, insomnia, anorexia and disorientation). The two chemists also complained of violent pain "sine materia in various organs" [14]. A more recent paper [21] describes chemical workers exposed to either high (n=12) or low (n=10) levels of TMT. The major symptoms reported by these investigators were forgetfulness, fatigue and weakness, headaches, attacks of rage and temper coupled with bouts of depression, and dimming of vision.

Although quantitative demonstration are lacking, rats exposed to TMT have been described as aggressive and difficult to handle [4,11]. Group housed animals often mutilated and destroyed each other, while individually housed rats engaged in self-mutilation. Unlike the self-mutilation which occurs in morphine addiction [19], these episodes were largely restricted to the tail. Furthermore, a single dose of TMT often produced episodes of seizures beginning 3–5 days later and lasting for about a week.

At least some of the constellation of symptoms described above suggest limbic system damage. This suggestion has been borne out by neuropathological studies in laboratory animals [3, 4, 5, 8]. Therefore efforts to validate neurophysiological methods for detection of neurotoxicity produced by TMT began with measures of the functional integrity of the limbic system.

One method for investigating the functional integrity of the limbic system is the kindling paradigm. Most areas of the limbic system, when repeatedly stimulated (i.e. once/day for several weeks), generate behavioral seizures. The process by which local EEG afterdischarges become behavioral seizures is called kindling [20], and the rate at which animals kindle has been used to characterize the response of the brain to a variety of toxicants (e.g. [18]). The kindling process in the hippocampus and the amygdala was examined in rats treated with TMT [12]. In the amygdala kindling experiment, rats were implanted with electrodes in the amygdala and treated on day 0 with a single dose of either 0, 5 or 7 mg/kg TMT. Electrical stimulation of the amygdala was administered at twice the threshold for production of a local afterdischarge (AD), and occurred once/day for 16 consecutive days. The results of the study, which are shown in Fig. 1 demonstrated that the treated rats kindled more rapidly than the controls. In the hippocampal kindling experiment the rats were implanted with electrodes in the dorsal hippocampus, and treated on day 0 with a single dose of either 0, 4, 5 or 6 mg/kg TMT. Since hippocampal kindling takes longer than amygdaloid kindling, the rats in this study were stimulated

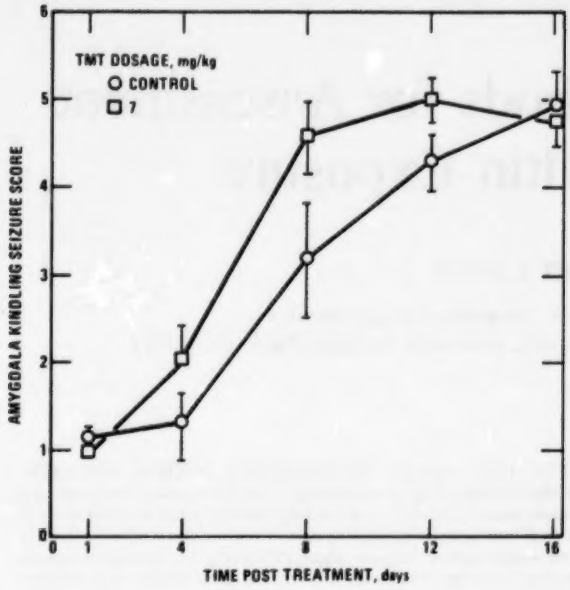

FIG. 1. Effects of TMT upon kindling rate following electrical stimulation of the amygdala in rats [12]. TMT was injected on day 0 only. Scores on the ordinate are from a scale developed by Racine [20] for rating seizure intensity, where higher scores indicate more severe seizures.

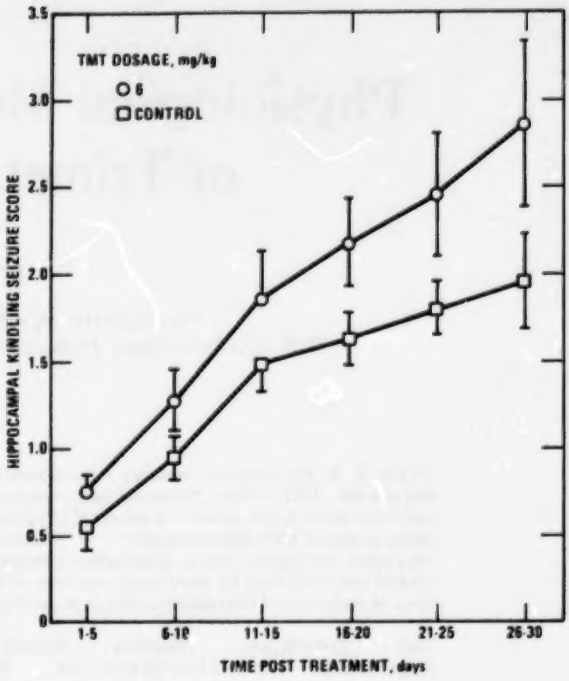

FIG. 2. Effects of TMT upon kindling rate following electrical stimulation of the dorsal hippocampal formation in rats [12]. Seizure scores were averaged into blocks of 5 days each. TMT was injected on day 0 only.

for 30 days instead of 16. Otherwise the procedures were identical. Again, the treated animals kindled somewhat more rapidly than the controls (see Fig. 2).

Studies with other compounds suggested that analysis of EEG afterdischarge properties following hippocampal stimulation are also useful for detecting toxicity [7,10]. These studies may be more rapidly performed than kindling studies, and provide potentially more information because more parameters are measured. Consequently rats implanted with electrodes in the dorsal hippocampus were studied following treatment with either 0, 5, 6 or 7 mg/kg TMT. A variety of properties of afterdischarges were studied on days 1, 4, 8 and 16 following treatment, compared to pretreatment control recordings. The results were surprising since the only variable which seemed to be affected was the threshold for production of the afterdischarge. None of the microproperties of the AD were changed [12]. Based upon this finding, a retrospective look was taken at the difference between before- and after-treatment AD threshold values obtained during the kindling experiments. The findings were similar: treatment with TMT increased the threshold for elicitation of an AD. These data are summarized on Fig. 3.

These data produced the puzzling conclusion that even though TMT reduced the number of cells, it did not disrupt the pattern of cell firing as detected by the AD procedure. The kindling data seemed to indicate that TMT increased limbic sensitivity to seizure development once activity was triggered. The sensitivity of the tests in detecting limbic dysfunction was disappointing. Alterations observed were not at levels substantially below those at which frank behavioral disturbances (e.g. self-mutilation) were observed. In an effort to increase the sensitivity of measures of limbic system function to damage, a somewhat more mechanistic approach, the use of intrahippocampal evoked potentials, is now under exploration.

Kainic acid produces a pattern of hippocampal damage similar in some respects to that produced by TMT (e.g. [22]). Since kainic acid-induced damage may result from overactivity of the mossy fiber system [22], an investigation of the functional integrity of the system which activates the mossy fibers was begun. In these studies the major input to the dentate granule cells, the perforant path, was electrically stimulated, and the resulting evoked potential was recorded from the granule cells. The output of the granule cells is a reflection not only of the perforant path input, but also of the status of other inputs, including recurrent inhibition produced by basket cells around the axon hillocks of the mossy fibers. There is a direct way for evaluating the functional integrity of recurrent inhibition in this system. It has been shown by Andersen *et al.* [2] that stimulation of the perforant path with a pair of pulses, each of which is sufficient to excite the granule cells, produces a reduced spike amplitude of the second response if the second stimulus follows the first within a particular period of time (20–40 msec). This time period is considerably greater than the refractory period of the spikes and the reduction in amplitude is therefore taken to directly reflect recurrent inhibition. Figure 4 illustrates this phenomenon.

To study the influence of TMT on recurrent inhibition in

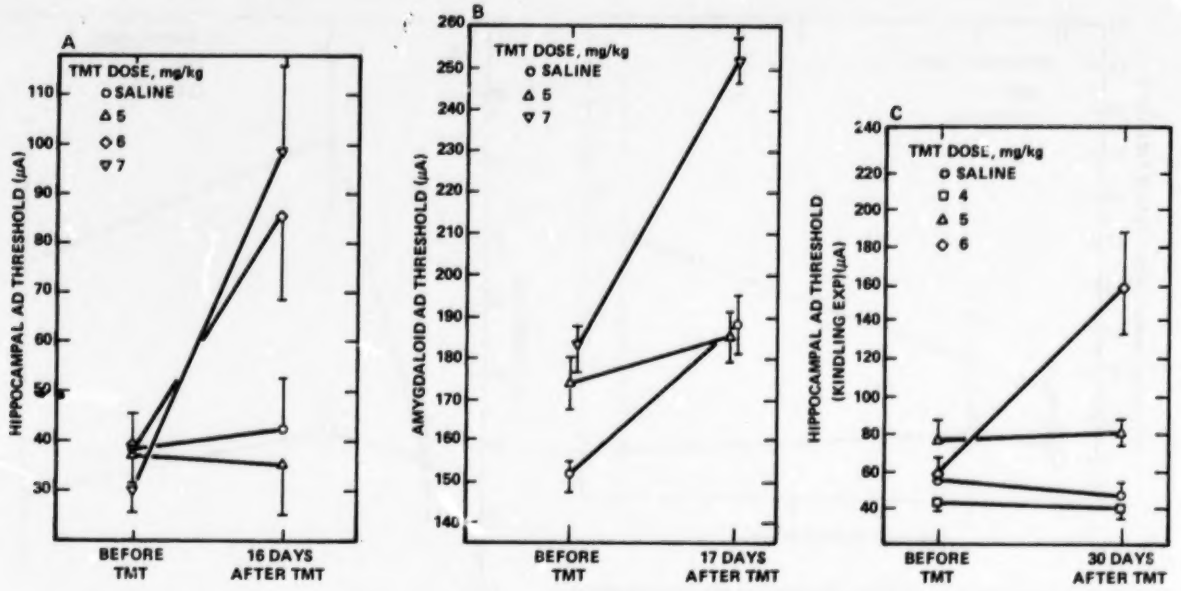

FIG. 3. Effects of TMT upon electrical thresholds for elicitation of an epileptiform afterdischarge from either (A) the hippocampal formation during an afterdischarge experiment; (B) the amygdala during a kindling experiment; or (C) the hippocampal formation during a kindling experiment.

FIG. 4. Paired pulse evoked potentials recorded from the dentate gyrus of a control rat following electrical stimulation of the perforant pathway. Each stimulus artifact (indicated by an arrow) is followed by an EPSP, which is interrupted by a population spike (marked with *) indicating discharge of the dentate granule cells. Time between the two stimuli is 20 msec, and the decreased amplitude of the second spike compared to the first is taken as a reflection of recurrent inhibition.

the dentate gyrus, the perforant path stimulating and dentate gyrus recording electrodes were implanted about two weeks prior to the onset of testing. These studies are still in progress, but to date the granule cell responses from treated and control animals have been recorded before and at several time points following treatment. Over the course of 3 days following a single treatment, the threshold for producing either an EPSP or a spike has not changed, nor do there appear to be significant differences between the spike amplitudes of treated and control rats. However the magnitude of recurrent inhibition appears to have been significantly decreased by the TMT treatment [13], and this effect appears to have begun within about 2 hrs of treatment (Fig. 5). This finding is similar to what has been discovered with kainic acid, and further reinforces the similarity between these two compounds [23]. These results and those obtained from studies of kainic acid raise the distinct possibility that the morphological damage produced by both agents is at least in part secondary to physiological dysfunction [13,22].

The rapid nature of the changes observed using the hippocampal recurrent inhibition measurements suggests that this method may be more useful than the kindling or AD methods in detection of TMT toxicity. Although changes are observed more rapidly with this technique than others, it remains to be determined whether changes may be observed at lower exposure levels.

Not all of the symptoms displayed by humans exposed to TMT can be accounted for by limbic system pathology. The aches, pains, and dimness of vision in humans, and the self-mutilation in rats might all reflect concurrent sensory disturbances. To provide a more complete picture of physiological dysfunction following TMT exposure, several recent studies have investigated sensory systems. The first of these

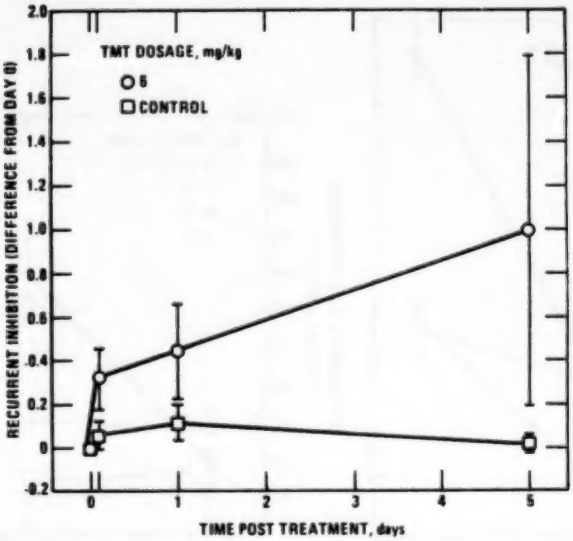

FIG. 5. Influence of 6 mg/kg TMT administered PO upon recurrent inhibition in the dentate gyrus. Recurrent inhibition is estimated by dividing the spike amplitude obtained from the second response of the pair by the spike amplitude obtained from the first response. The ordinate reflects Day X-Day 0. Thus negative numbers indicate more inhibition than on Day 0, and positive numbers indicate less inhibition than on Day 0. TMT reduced recurrent inhibition beginning within 2 hrs of dosing.

studies investigated the visual system since alterations in the visual evoked potential often reflect general central nervous system dysfunction [6]. Based upon the absence of demonstrated pathology in the retina or other areas of the visual system [4], it was initially hypothesized that the visual evoked potential would not detect TMT-induced damage.

To study the visual system rats were implanted with electrodes in the visual cortex or optic tract. Flash-evoked potentials were recorded immediately before exposure, and at intervals of 1, 4, 8 and 16 days following exposure. The findings did not support the predicted lack of effect. Latencies to the early peaks of the evoked potential were increased, and the fact that this occurred with roughly equal magnitude in both the cortically recorded and optic tract recorded potentials indicated that the dysfunction was probably of retinal origin [9]. Among the more interesting features of this finding was its time course. Unlike most of the behavioral consequences of TMT exposure, the effects on the visual system were present within 24 hrs and persisted for the entire test period (Fig. 6). The reports of no retinal pathology [4] were perhaps premature. A more recent report from the same laboratory [11] describes damage in both inner and outer nuclear layers of the marmoset retina following exposure to TMT. These findings may be related to the dimness of vision reported by the humans exposed to TMT.

A similar although less extensive study has been performed to investigate the effects of TMT upon the somatosensory system. Based upon observations of self-mutilation of the tail, it was hypothesized that denervation might have occurred. To examine this possibility the tail was

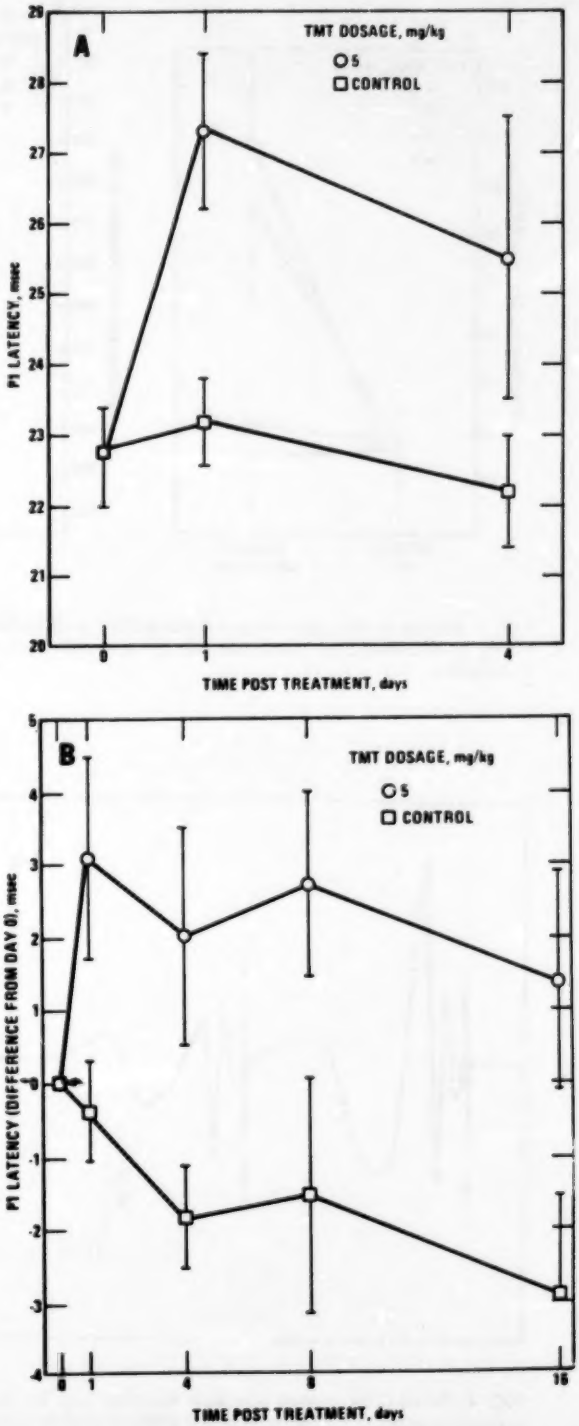

FIG. 6. Effects of TMT upon potentials evoked in the visual system of the rat. A. Effects of TMT upon latency of the first positive wave recorded from the optic nerve; B. Effects of 5 mg/kg TMT upon latency of the first positive wave recorded from the visual cortex. Values plotted are differences from pre-exposure levels.

electrically stimulated and the somatosensory evoked potential was recorded from the somatosensory cortex. The alterations observed were not as extensive as they were in the visual system, but nevertheless dysfunction could be detected in the TMT-exposed rats [16]. TMT increased latencies and decreased amplitudes, just as it did in the visual system. Because of the relatively minor changes observed, the data were interpreted as not supporting a denervation explanation of the tail mutilation, and this phenomenon therefore remains unexplained.

Systematic studies of auditory function following TMT administration have not been published. However the presence of dysfunction in the visual and somatosensory systems raises the possibility of a general sensory system defect. Some data are available to support extension of this generality to the auditory system. In a pilot study rats were tested in an auditory startle task immediately before and for several days following TMT exposure. There was a severe impairment of the response [15]. An assessment of the startle response in grasshopper mice [17] also demonstrated response amplitude suppression induced by TMT exposure. These deficits may, of course, reflect a motor impairment, although on the grounds of parsimony auditory dysfunction is more probable. Regardless of the interpretation of the startle data, it is quite clear that TMT produces sensory deficits in addition to limbic system dysfunction.

The above data indicate that TMT is not nearly as selective as a cursory glance at the pathology might suggest. Based upon the sensory system data and the spontaneous seizures which occur at high dosages, the possibility that TMT produces generalized changes in CNS excitability was investigated with the systemic convulsant pentylenetetrazol (PTZ). Rats were treated with TMT and 5 days later challenged with PTZ. The results supported the hypothesis by indicating that the TMT-treated rats were more likely to convulse than untreated control rats [12] when both were administered a dosage of PTZ [12].

CONCLUSIONS

Several important conclusions may be drawn from this series of experiments. First, while there are many physiological methods by which the functional integrity of the limbic system may be evaluated, the one which appears to give the most direct information and is perhaps the most cost effective is the hippocampal evoked potential.

Second, the existence of obvious regional pathology following exposure to a toxicant should not lead to the assumption that there is not functional impairment elsewhere. The sensory dysfunctions following exposure to TMT would not have been discovered for some time if the physiology experiments awaited prior demonstration of pathological damage to these systems.

Finally, it is extremely difficult to generalize between the functional consequences of closely related organotin compounds. These experiments, when compared to those which have concentrated on triethyltin, demonstrate how poorly structure activity relationships are understood in neurotoxicology.

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